DEVELOPMENT OF A BACTERIAL EXPRESSION SYSTEM FOR PRODUCTION OF β-LACTAMASE

Project report submitted to Sister Nivedita University in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE
IN
MICROBIOLOGY

Submitted By

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TO WHOM IT MAY CONCERN

This is to certify that **Ms. Rupsa Kundu**, a student of the Department of Microbiology, Sister Nivedita University, has successfully completed her long-term training at **ATGC Diagnostics Pvt. Ltd.**,West Bengal, India, in the domain of Molecular Biology, from **4th January 2025 to 16th May 2025.**

During this period, she was engaged in a research project titled **"Development of a bacterial expression system for production of β-lactamase,"** under the supervision of **Dr. Siddhartha Roy**, Principal Investigator (PI) of the project. The project involved the design and development of a recombinant system for the expression of the β-lactamase enzyme in bacterial hosts. Ms. Kundu was trained in and performed various core molecular biology techniques including restriction digestion, ligation, plasmid isolation, bacterial transformation, recombinant protein expression, and SDS-PAGE analysis. She also gained exposure to the principles of gene cloning, vector selection, and expression optimization.

Ms. Kundu demonstrated a high level of enthusiasm, responsibility, and dedication throughout the training. Her ability to grasp new techniques quickly and apply them effectively in the laboratory was commendable. She maintained professionalism and contributed positively to the lab environment, both individually and as a team member.

We appreciate her efforts and wish her continued success in her academic and professional pursuits.

28/05/2025

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TO WHOM IT MAY CONCERN

This is to certify that the project report titled **"DEVELOPMENT OF A BACTERIAL EXPRESSION SYSTEM FOR PRODUCTION OF β-LACTAMASE"**, submitted by Ms. Rupsa Kundu (Registration No. 230010084052, Enrollment No. 2322211001008) to the department of Microbiology, School of Health Science and Translational Research at Sister Nivedita University, Kolkata, is a bona fide record of research conducted by her for the partial fulfilment of the requirements for the Master of Science degree.

Date: 5th June, 2025

Place: Newtown, Kolkata

Signature of Head of the Department

DECLARATION BY THE STUDENT

I hereby declare that the work which is being presented in this dissertation entitled **"Development of a bacterial expression system for production of β-lactamase"** in partial fulfilment of the requirements for the award of the degree of **Masters of Science** in **Microbiology** is an authentic record of my own work carried out during the period from **2nd January to 2nd May** under the supervision of **Dr. Siddhartha Roy, Director of ATGC diagnostics Pvt.Ltd.**

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Rupsa Kundu
05.06.2025

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ABSTRACT

A key component of contemporary biotechnology is the creation of recombinant proteins using bacterial expression systems, which provide scalable and reasonably priced platforms for therapeutic uses. In the study, we developed a bacterial system for the expression of β-lactamase, an enzyme known not only for conferring antibiotic resistance but also for its emerging potential in therapeutic interventions, such as protecting gut microbiota during antibiotic treatment.

The bla gene encoding β-lactamase was selectively amplified from the pUC19 cloning vector using a pair of synthetic primers designed to include EcoRI and HindIII restriction sites, enabling directional cloning. Initial amplification attempts with supercoiled pUC19 DNA were unsuccessful, likely due to reduced primer accessibility. The target ~800bp segment was amplified efficiently by PCR after pUC19 was digested to linear form. The purified PCR product was digested with appropriate restriction enzymes and ligated into the pET-28a expression vector, which provides a T7 promoter for high-level transcription and a His-tag for potential purification. The ligation product was transformed into *E. coli BL21(DE3)*, a strain engineered for T7 polymerase- mediated expression. Transformants were selected on kanamycin agar plates and subsequently re-screened on ampicillin for dual-selection verification. By means of restriction enzyme digestion and plasmid isolation, recombinant colonies were verified. Confirmed clones were cultured, and protein expression was induced using 1mM IPTG at mid-log phase (OD_{600} 0.6-0.8). After induction, cells were harvested and lysed, and the protein extract was denatured with Laemmli buffer at 95°C to unfold proteins. Successful expression was confirmed by SDS-PAGE analysis, which showed a clear band at about 31kDa, which is the molecular weight of β-lactamase. The project demonstrates the successful construction and functional verification of a recombinant expression system for β-lactamase in *E. coli*. The methodology used serve as a foundational approach for the production of therapeutic proteins in prokaryotic hosts, with future potential for purification, characterization, and downstream biomedical application.

KEYWORD

β-lactamase, Therapeutic protein, Recombinant expression, pUC19, pET-28a, *E. coli BL21(DE3)*, PCR amplification, IPTG induction, SDS-PAGE, Molecular Cloning, Antibiotic Resistance.

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ABBREVIATIONS

Abbreviation	Full form
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
PCR	Polymerase Chain Reaction
IPTG	Isopropyl β-D-1-thiogalactopyranoside
SDS-PAGE	Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
LB	Luria-Bertani
OD	Optical Density
bp	Base pairs
kDa	Kilodaltons
RE	Restriction Enzyme
bla	β-lactamase
pUC19	A high copy plasmid used for cloning
pET-28a	Plasmid for expression by T7 RNA polymerase
DE3	A lysogen of *E. coli* containing T7 RNA polymerase
TAE	Tris-Acetate-EDTA
TBE	Tris-Borate-EDTA
EDTA	Ethylenediaminetetraacetic Acid
Tris	Tris (hydroxymethyl) aminomethane

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INTRODUCTION

Recombinant DNA technology has greatly revolutionised biotechnology by allowing microbial synthesis of therapeutic proteins. Among microbial hosts, *Escherichia coli* is extensively used due to its rapid proliferation, well-understood genetics, and ability to express diverse foreign proteins. Utilizing bacterial expression systems offers multiple benefits, including scalability, low production costs, and ease of genetic manipulation. This study aims to establish a bacterial platform for the recombinant expression of β-lactamase, which serves both as a model enzyme and a potential therapeutic candidate.

β-lactamase is an enzyme capable of breaking down β-lactam antibiotics, such as penicillin and cephalosporin, rendering them ineffective. Although commonly associated with antibiotic resistance, recent findings indicate that β-lactamase can be repurposed for therapeutic use. For instance, it may help preserve the gut microbiome during antibiotic treatment by neutralizing residual antibiotics in the gastrointestinal tract. Given this therapeutic potential, β-lactamase was selected as the target protein for expression in a bacterial host.

The gene encoding β-lactamase (bla) is naturally carried by the pUC19 plasmid—a frequently used vector in molecular cloning. In this work, the bla gene was amplified from pUC19 using specially designed primers that incorporated recognition sequences for the restriction enzymes EcoRI and HindIII. These locations were chosen in order to facilitate directional cloning into the pET-28a expression vector's multiple cloning site (MCS). To improve amplification efficiency, pUC19 was linearized by restriction digestion prior to PCR, mitigating the limitations posed by supercoiled DNA.

In order to create cohesive ends that were compatible with the digested pET-28a vector, the bla gene was isolated and digested using EcoRI and HindIII after PCR. A T7 promoter for robust, inducible expression and an N-terminal 6xHis-tag for simplicity of purification are features of this expression plasmid. The digested vector and insert were ligated using T4 DNA ligase and transformed into *E. coli BL21(DE3)* cells, which are optimized for T7 promoter-driven expression and inducible by IPTG.

Transformants were selected on LB agar plates containing kanamycin, as the pET-28a vector confers resistance to this antibiotic. Colonies underwent plasmid isolation and further screening on ampicillin-containing plates to confirm the inclusion of the bla gene. Restriction digestion of the extracted plasmids was conducted to verify the presence and correct orientation of the insert.

Recombinant strains confirmed to carry the bla gene were cultured, and protein expression was induced using 1 mM IPTG at mid-log phase (OD_{600} ~0.6–0.8). After induction, bacterial pellets were collected, lysed, and prepared for SDS-PAGE analysis. Laemmli buffer was used to denature the samples and heated at 95°C. A protein band corresponding to β-lactamase was observed at 31kDa, confirming successful expression.

In summary, this project demonstrates the successful development of a bacterial expression system for β-lactamase using *E. coli BL21(DE3)* and the pET-28a vector. This system not only provides a model for the production of recombinant therapeutic proteins but also lays the groundwork for future purification and functional characterization studies. The methodologies applied in this study, including primer design, directional cloning, and protein expression analysis, are foundational to molecular biology and biotechnological applications.

LITERATURE REVIEW

When genetic engineering and molecular cloning techniques are combined, the field of producing recombinant proteins has seen great breakthroughs. Among various host systems, *Escherichia coli* remains the most widely used organism for the production of recombinant proteins due to its simple cultivation, rapid growth, and well-studied genetics. The ability to express cloned genes in *E. coli* has provided an essential platform for producing therapeutic, industrial, and research-grade proteins. One of the fundamental aspects of recombinant protein production involves the use of expression vectors that can efficiently regulate gene transcription and translation. The pET-28a system, is a widely utilized expression vector that contains the T7 promoter for high-level expression and a 6x His-tag for easy protein purification through nickel affinity chromatography. A host strain such as *E. coli BL21(DE3)*, which carries the T7 RNA polymerase gene under lac promoter control, is necessary for this approach. Induction with isopropyl β-D-1-thiogalactopyranoside (IPTG) triggers the expression of the gene cloned downstream of the T7 promoter.

β-lactamase, the target protein in this project, is a bacterial enzyme known for its ability to hydrolyze β-lactam antibiotics, thereby conferring resistance to the producing organism. While traditionally associated with antimicrobial resistance, β-lactamase has garnered interest for its possible therapeutic applications. Studies have suggested that orally administered β-lactamase can inactivate antibiotics in the gut, potentially protecting commensal microbiota from disruption during antibiotic therapy (Ubeda et al., 2013). This functional versatility makes β-lactamase an interesting candidate for recombinant production and further functional exploration also for screening of β-lactamase inhibitors. The source of the β-lactamase gene in this study is the pUC19 plasmid, a high-copy-number cloning vector commonly used in molecular biology laboratories. It has the bla gene, which confers ampicillin resistance and encodes β-lactamase. The high-copy nature of pUC19 ensures ample quantities of plasmid DNA for downstream applications such as gene amplification.

Molecular cloning relies heavily on the polymerase chain reaction (PCR) for gene amplification. In this project, primers were designed with restriction enzyme recognition sites for EcoRI and HindIII to facilitate directional cloning. Compatibility between the amplified

gene and the expression vector is ensured by include restriction sites in primers. Linearization of supercoiled plasmid DNA prior to PCR has been shown to improve amplification efficiency, as it provides better accessibility for the DNA polymerase to the target region.

Following amplification and digestion, the gene insert and vector are ligated using T4 DNA ligase. This enzyme catalyzes the formation of phosphodiester bonds between adjacent nucleotides, thereby creating a stable recombinant plasmid. The ligated product is then transformed into chemically competent *E. coli cells*, such as *BL21(DE3)*, for propagation and expression. Transformation efficiency and plasmid integrity are verified using antibiotic selection and plasmid isolation followed by restriction digestion analysis.

Upon confirming the presence of the recombinant construct, the transformed cells are cultured and induced with IPTG to initiate protein expression. Protein synthesis results from the transcription of the target gene by the T7 RNA polymerase during induction. The expressed proteins are typically harvested from the cell pellet, denatured using Laemmli buffer, and analyzed using SDS-PAGE. This technique separates proteins based on molecular weight, allowing visualization of the expressed β-lactamase as a distinct band at approximately 31kDa. Previous studies have established the efficiency of E. coli systems for expressing a variety of therapeutic proteins, including β-lactamase. Research by (Banerjee et al. 2012) demonstrated successful expression and purification of recombinant β-lactamase, highlighting the reliability of the pET-28a system and the importance of expression condition optimization. These findings provide a strong foundation for using bacterial systems in both research and therapeutic protein production.

In conclusion, the literature supports the selection of *E. coli* as a host organism, the use of pET-28a as an expression vector, and the utility of β-lactamase as a model therapeutic protein. The integration of these components in the present study is well-founded and represents a standard yet effective approach in recombinant protein biotechnology.

METHODOLOGY

1. PRIMER DESIGNING PROCEDURE:

- The β-lactamase gene (**bla gene**) was efficiently cloned from pUC19 and pET-28a was used for expression by a meticulously planned primer creation approach. The NCBI primer designing tool was utilized to guarantee specificity and efficiency. The initial step consisted of choosing the target gene responsible for ampicillin resistance, which produces β-lactamase. The forward primer was selected from a previously published study work "PNAS March 19,2002 vol. 99 no. 6 3469-3474". Forward primer is 'ATGAGTATTCAACATTTCCGTGTCG'. I looked for a reverse primer that would complement and bind at the precise intended site, using NCBI primer designing tool. To enhance the cloning process, EcoRI and HindIII restriction sites were added to the 5' ends of the forward and reverse primers, respectively. This modification guaranteed that, following digestion, the gene could be inserted in the correct orientation into the multiple cloning site (MCS) of the vector without compromising critical sequences.

Primer pair 8

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	ATGAGTATTCAACATTTCCGTGTCG	Plus	25	17	41	59.94	40.00	6.00	2.00
Reverse primer	AGTAAACTTGGTCTGACAGTTACCA	Minus	25	896	872	59.87	40.00	5.00	2.00
Product length	880								

Fig1.1: Primer pair

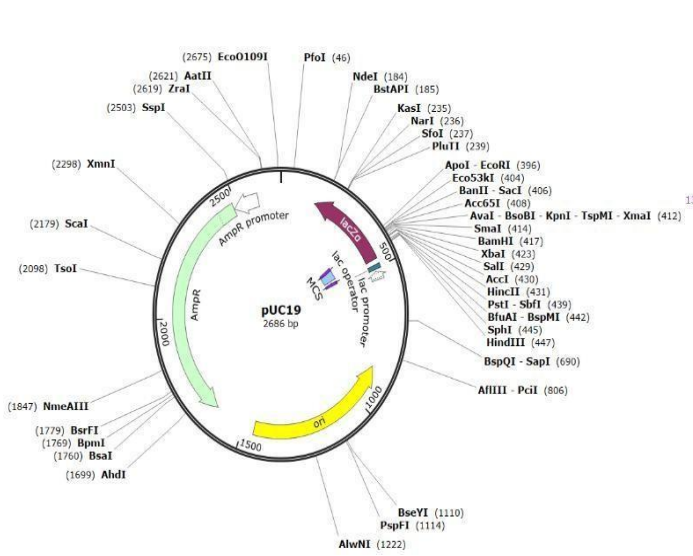


Fig1.2: pUC19 plasmid

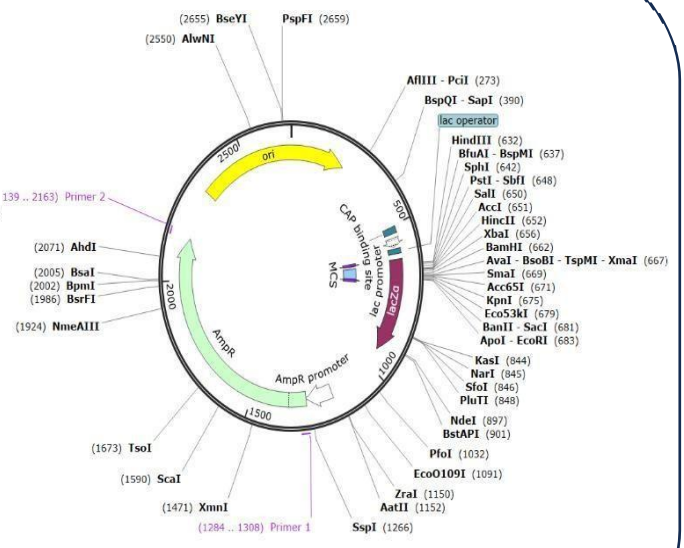


Fig1.3: designed pUC19 plasmid with primer

- After incorporating the restriction sites, the primers were fine-tuned for specificity, melting temperature (Tm), and GC content to facilitate effective binding and amplification during PCR. The GC content was kept between 40% and 60% to promote stable hybridization, while the melting temperature was set between 55°C and 65°C to limit primer-dimer formation and ensure reliable amplification. The primers were crafted to have lengths ranging from 18 to 25 base pairs, striking a balance between specificity and efficiency. To verify their accuracy, the primers underwent testing with Snap Gene, where their binding locations and the existence of the restriction sites were confirmed. This procedure was crucial in ensuring that the primers would amplify the intended region without causing any unintended secondary

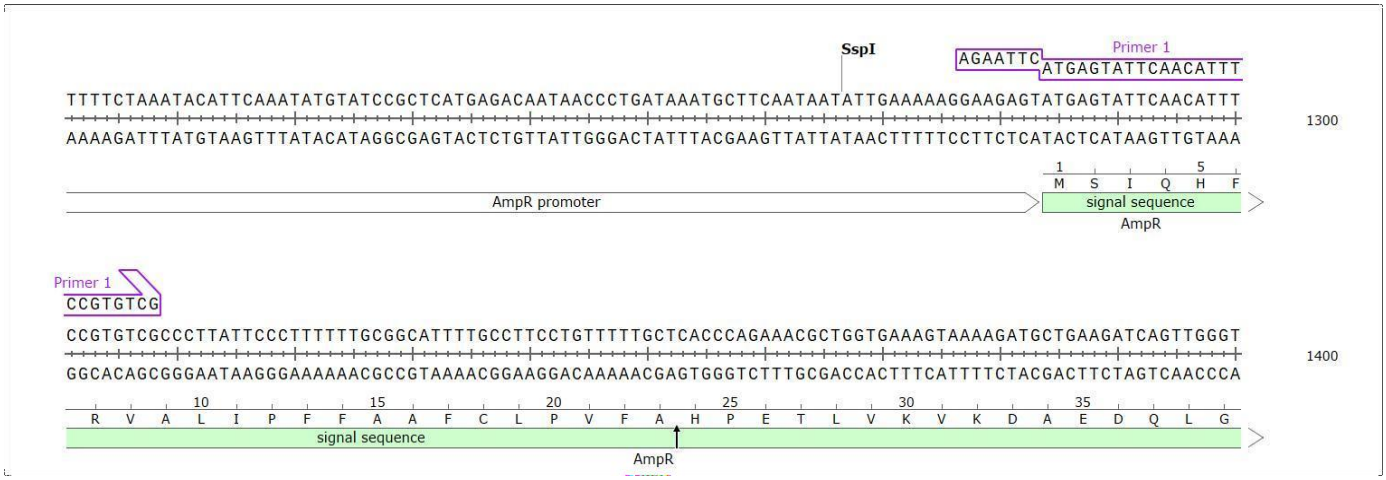


Fig1.4: Forward primer with designed EcoRI

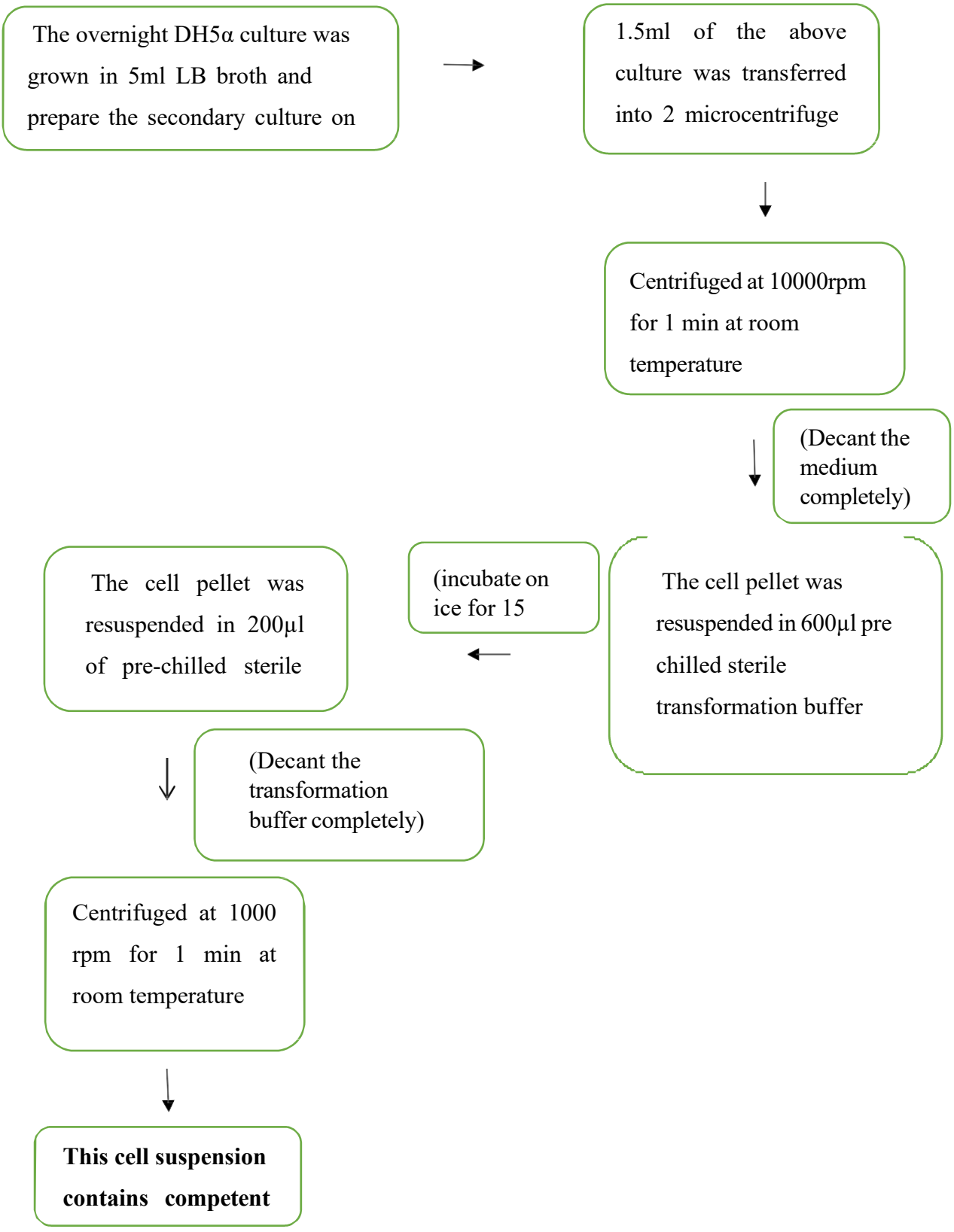
TCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATA
AGAGCGCCATAGTAACGTCGTGACCCCGGTCTACCATTCGGGAGGGCATAGCATCAATAGATGTGCTGCCCCTCAGTCCGTTGATACCTACTTGCTTTAT
2100
S R G I I A A L G P D G K P S R I V V I Y T T G S Q A T M D E R N
AmpR
2146
GACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTA
CTGTCTAGCGACTCTATCCACGGAGTGACTAATTCGTAACCATTGACAGTCTGGTTCAAATGAGTATATATGAAATCTAACTAAATTTTGAAGTAAAAAT
2200
R Q I A E I G A S L I K H W
AmpR
ACCATTGACAGTCTGGTTCGAATGA
Primer 2

Fig1.5: Reverse primer with designed HindIII

- After successful validation, the primers were obtained and dissolved in nuclease-free water to achieve a stock concentration of 100µM, with a working concentration of 10µM utilized in PCR reactions.

2. TRANSFORMATION OF Puc19 INTO *E. coli* DH5α:

- **COMPETENT CELL PREPARATION:**


The overnight DH5α culture was grown in 5ml LB broth and prepare the secondary culture on
1.5ml of the above culture was transferred into 2 microcentrifuge
Centrifuged at 10000rpm for 1 min at room temperature
(Decant the medium completely)
The cell pellet was resuspended in 600µl pre chilled sterile transformation buffer
(incubate on ice for 15
The cell pellet was resuspended in 200µl of pre-chilled sterile
(Decant the transformation buffer completely)
Centrifuged at 1000 rpm for 1 min at room temperature
This cell suspension contains competent

- **CELL TRANSFORMATION:**

pUC19 plasmid DNA was added in the competent cell

(Incubated tubes on ice for 10 min)

Transferred them to a preheated water bath set at a temperature of 42°C for 90 seconds.

(Rapidly transferred the tubes on ice for 5min)

800µl of LB broth was added to all the tubes. Incubated the tubes for 45 min at 37°C on a shaker at 140 rpm.

(centrifuged at 10000 rpm for 1 min at room temperature)

200µl of cell culture was plated from each tube on LB-amp agar plate & incubated at 37°C

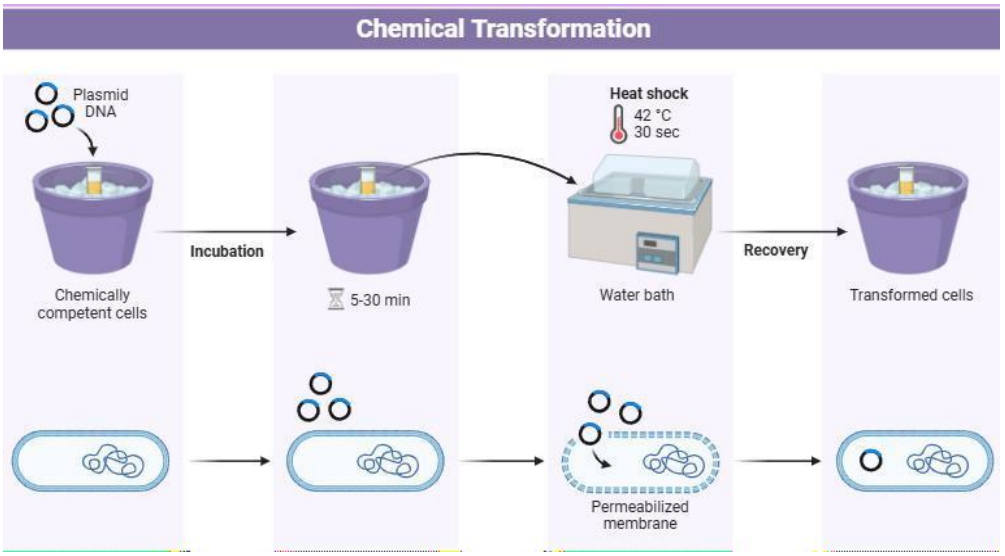


Fig2.1.: cell transformation

3. PLASMID CONFIRMATION:

Picked up the single isolated colony from pUC19 plate and grown overnight in 5ml LB broth containing ampicillin for plasmid preparation.

↓

1.5ml of above culture was transferred into microcentrifuge tubes and centrifuged at 10000 rpm for 3 min at room temperature.

↓

250µl P1 solution was added

↓ (discard flow through)

350µl of P2 solution was added and invert mixing

↓ (Centrifuge at 10000 rpm for 10 min at RT)

Supernatant was taken into the column and centrifuge for 1min

↓

500µl PW1 solution was added and centrifuged at 10000 rpm for 1 min at RT

↓ (discard flow through)

700µl PW2 solution was added and centrifuged at 10000 rpm for 1 min at RT

↓

Free spin for 1 min at 10000 rpm at RT. And a fresh tube was taken and placed the column.

↓

50µl nuclease free water was added and rest in 5 min. then centrifuged it at10000 rpm for 1 min. Total 50µl plasmid DNA was prepared.

↓

pUC19 plasmid was digested with EcoRI and HindIII enzyme to linearize and see in the gel electrophoresis for plasmid confirmation

4. PCR AMPLIFICATION:

After designing specific primers with EcoRI and HindIII restriction sites, gene amplification was performed to isolate the β-lactamase (ampicillin resistance) gene from the pUC19 plasmid. The initial PCR attempt using the intact pUC19 plasmid as the template did not yield successful amplification. This may have been due to secondary structures or inaccessibility of the target region within the supercoiled plasmid.

To overcome this issue, the pUC19 plasmid was first digested with EcoRI and HindIII to linearize the DNA and expose the gene of interest. This linearized plasmid was then used as a template for PCR, which successfully resulted in amplification of the expected ~880 bp β-lactamase gene. The PCR product was visualized on agarose gel, confirming the correct band size and the success of the amplification step.

Table 4.1: PCR cycle

step	**Temperature**	**Time**
Initial Denaturation	95°C	5 min
Denaturation	95°C	30 sec
Annealing	60°C	30 sec
Extension	72°C	53 sec
Final Extension	72°C	10 min
Hold	25°C	1min

5. PREPARATION OF INSERT:

After confirming successful amplification, the PCR product containing the bla gene was purified using a PCR purification kit to remove primers, nucleotides, enzymes, and salts. The purified product was then digested with EoRI and HindIII restriction enzymes to generate compatible ends for cloning. The digestion mixture was incubated under optimal conditions (typically at 37°C for 1 hour). Following digestion, the product was again purified using a PCR purification kit to isolate the digested fragment. This purified, double digested insert was ready for ligation into the expression vector.

Flowchart

393ng(130ng/µl) pUC19 plasmid DNA was transformed into *E. coli* DH5α

↓

After transformation and final centrifugation, 200µl of this mixture was plated on ampicillin resistant plate.

↓

Single isolated colony was picked and inoculated into LB broth containing ampicillin for plasmid preparation.

↓

Plasmid DNA was extracted from overnight culture and 726ng(242ng/µl) DNA of the prepared plasmid was analyzed on 1% agarose gel to confirm the presence of

↓

For confirmation of plasmid, restriction digestion of pUC19. 484ng(242ng/µl) of plasmid DNA was used in a 20µl total reaction volume.

↓

Digestion was successful, as confirmed by gel electrophoresis.

↓

PCR amplification with digested pUC19. (conc. 409.5ng/µl)

↓

PCR product purification.

↓

Digestion of PCR product with EcoRI and Hind III.

↓

PCR product purification (Insert prepared, conc. 130ng/µl).

PCR purification procedure (SLS' PCR clean-up kit)

5 volumes of buffer PW1 was added to 1 volume of the PCR sample and mixed it properly.

↓

To bind DNA, the sample was applied to the column, and centrifuged for 1 min at ≥ 10000 rpm.

↓

Flow through was discarded and placed column back in the same collection tube.

↓

To wash, 0.75ml buffer PW2 was added to the column and centrifuged for 1 min at ≥10000 rpm.

↓

Flow through the discarded and placed the column back in the same tube. Centrifuged the column for an additional 1min at≥ 10000 rpm.

↓

The column was placed in a clean new 1.5ml micro centrifuge tube.

↓

To elute DNA, 50µl buffer PE was added to the center of the membrane and centrifuge the column for 1 min. alternatively, for increased DNA conc., 30µl elution buffer was added to the center of the membrane, let the column strand for 1 min, and then centrifuged for 1min at≥10000 rpm.

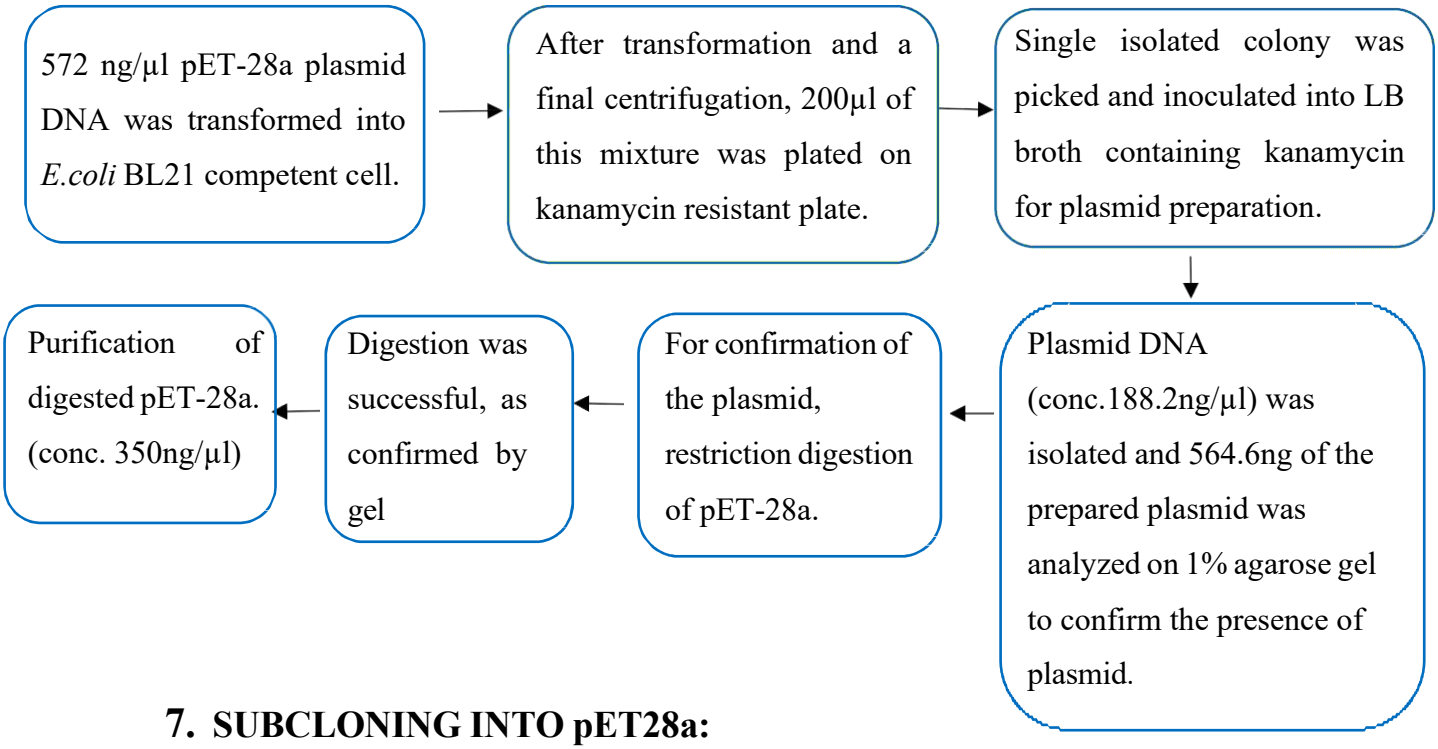
↓

Then the DNA sample (Insert, conc.130ng/µl) was stored at -20°C.

6. PREPARATION OF EXPRESSION VECTOR (pET-28a):

The pET-28a vector was propagated in *E. coli BL21(DE3)* cells and plasmid DNA was extracted following by the previous method. In order to produce compatible sticky ends for ligation with the insert, the vector was digested with EcoRI and HindIII. The linearized vector was purified using a PCR purification kit same as before.

Flowchart


572 ng/µl pET-28a plasmid DNA was transformed into *E.coli* BL21 competent cell.
After transformation and a final centrifugation, 200µl of this mixture was plated on kanamycin resistant plate.
Single isolated colony was picked and inoculated into LB broth containing kanamycin for plasmid preparation.
Plasmid DNA (conc.188.2ng/µl) was isolated and 564.6ng of the prepared plasmid was analyzed on 1% agarose gel to confirm the presence of plasmid.
For confirmation of the plasmid, restriction digestion of pET-28a.
Digestion was successful, as confirmed by gel
Purification of digested pET-28a. (conc. 350ng/µl)

7. SUBCLONING INTO pET28a:

T4 DNA ligase was used to ligate the digested pET-28a vector and the purified, digested bla inser. The ligation reaction was incubated at optimal conditions, and the product was transformed into competent *E. coli BL21*(DE3) cells. Transformations were selected on kanamycin-resistant LB agar plates.

8. DOUBLE SELECTION SCREENING:

Colonies that grew on kanamycin plates were re-plated on ampicillin-resistant plates. Growth on both antibiotic plates confirmed the successful integration of the bla-gene. Positive colonies were selected for plasmid isolation.

9. CONFIRMATION OF CLONING:

Plasmid DNA from positive colonies was digested with EcoRI and HindIII to verify the presence of the insert. Gel electrophoresis revealed two bands, corresponding to the vector backbone (~5.3kb) and the insert (~880bp).

10. PROTEIN EXPRESSION VIA IPTG INDUCTION & SDS-PAGE ANALYSIS:

In this experiment, *E. coli BL21(DE3)* cells carrying the recombinant pET-28a plasmid containing the bla gene were used to express proteins. The bacterial culture was grown in LB broth with both kanamycin and ampicillin until it reached an optical density (OD_{600}) of approximately 0.6–0.8, indicating the mid-log phase of growth. At this stage, 1 mM and 0.5 mM IPTG were administered to activate the expression of the β-lactamase gene regulated by the T7 promoter.

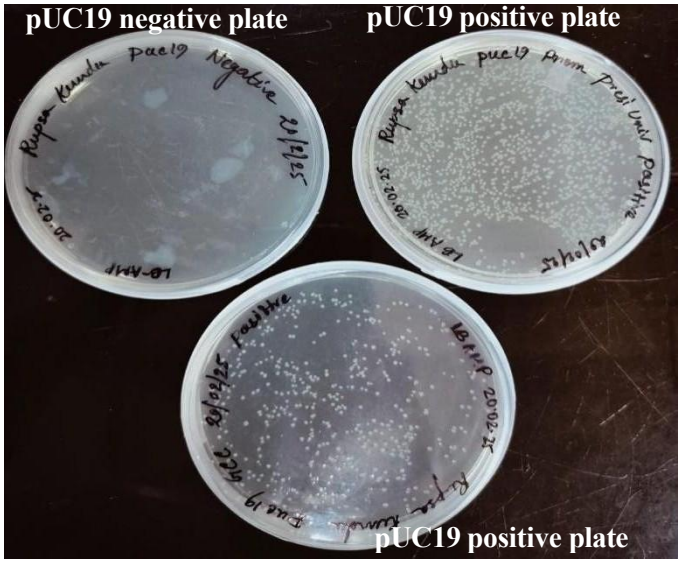
Following induction, the culture was incubated for 4 hours to allow sufficient protein production. Cells were then harvested by centrifugation, and the resulting pellet was resuspended in Laemmli buffer. The sample was heated at 95°C to denature the proteins and ensure uniform unfolding before analysis.

The expressed protein was analyzed by SDS-PAGE, a technique that separates proteins based on molecular weight. A clear and distinct band corresponding to approximately 31kDa was observed, which matched the expected size of β-lactamase. This result confirmed that the bla gene was successfully expressed in the bacterial system and that the recombinant protein was present in the cell lysate.

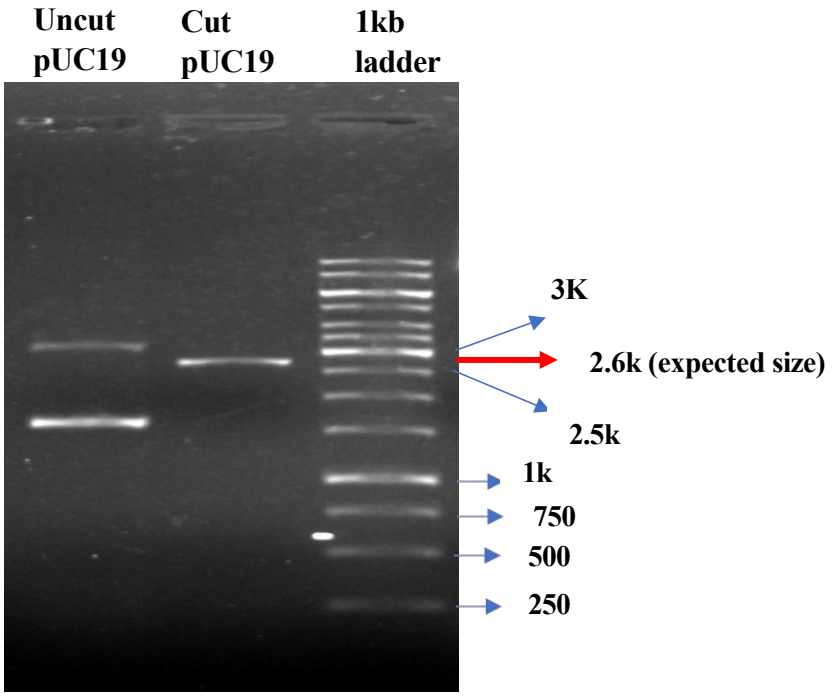
RESULT

A. TRANSFORMATION OF pUC19 INTO *E. Coli DH5α* CELL:

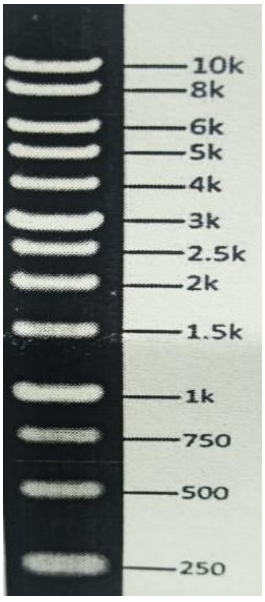
White colonies appeared on LB agar plates containing ampicillin, indicating successful transformation of *E. coli DH5α* with the pUC19 plasmid. No colonies were observed on the control plate. Plasmid was extracted from chosen colonies and underwent restriction digestion. Agarose gel electrophoresis showed bands corresponding to the expected size of pUC19, confirming the presence of the plasmid.



FigA.1: pUC19 plate



FigA.2.: confirmation of pUC19 plasmid



FigA.3.: 1% Agarose Gel

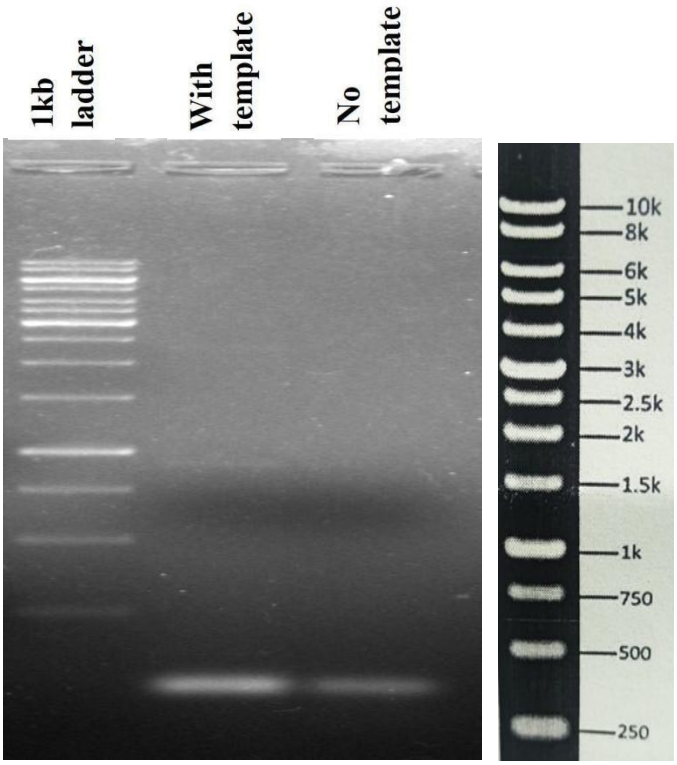
B. PCR AMPLIFICATION:

TableB.1: Restriction Digestion

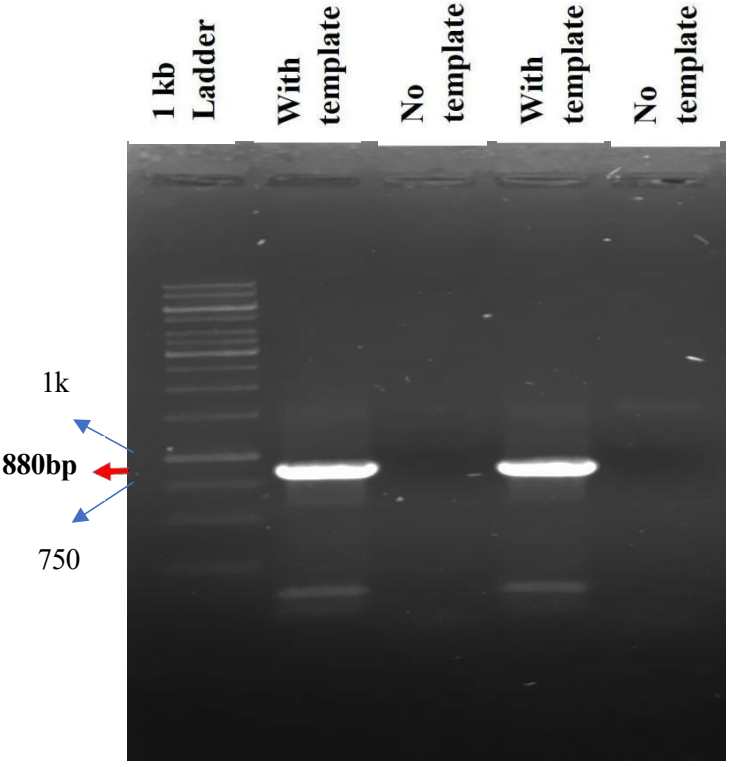
Reagent	Amount
pUC19 DNA	2µl (338ng/µl)
Enzyme (EcoRI & HindIII)	0.5µl
Tango Buffer	2µl
Nuclease Free Water	15µl
Total	20µl

TableB.2: PCR Reagent

PCR reagent	Amount
DNTP	2µl
PCR buffer(10X)	2.5µl
Reverse Primer(10µM)	0.5µl
Forward Primer(10µM)	0.5µl
Taq polymerase	0.25µl
Template DNA (digested DNA)	1µl (16.9ng/µl)
Nuclease Free water	18.25µl
Total	25µl



FigB.3.: Initial failure due to supercoiled plasmid



FigB.4.: Successful amplification (Expected size ~880bp)

C. LIGATION

- *Insert (PCR product) preparation:*

1. Digest 780ng of PCR amplified DNA with EcoRI & HindIII for 2 hours at 37°C.

780ng(130ng/µl)	- pUC19 DNA
1µl	- EcoRI enzyme (10 units/µl)
1µl	- HindIII enzyme (10 units/µl)
3µl	- Tango buffer (10X)
19µl	- Nuclease Free Water
30µl	- Total Volume

2. Purify the digested plasmid using PCR purification kit.

- *Vector(pET28a) preparation:*

1. Digest 1128ng(188.2ng/µl) of pET28a with EcoRI & HindIII for 2 hours at 37°C.

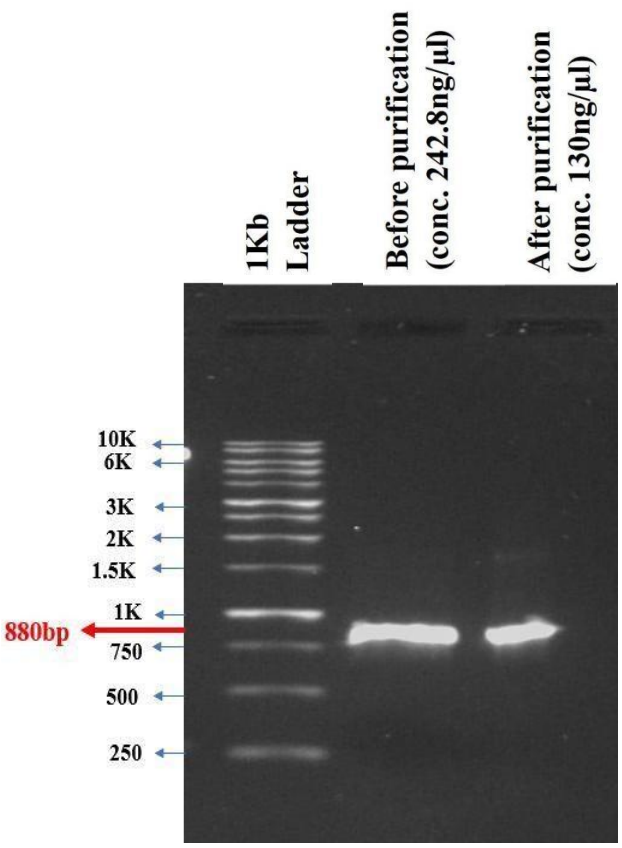
1128ng(188.2ng/µl)	- pET28a DNA
1µl	- EcoRI (10 units/µl)
1µl	- HindIII (10 units/µl)
3µl	- Tango Buffer (10X)
19µl	- Nuclease Free Water
30µl	- Total Volume

2. Purify the digested pET28a plasmid using PCR purification kit.

❖ Set up ligation reaction and incubated at 16°C for overnight.

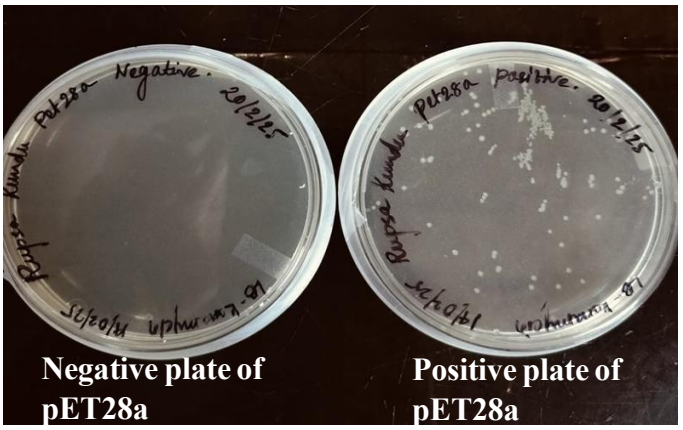
700ng(350ng/µl)	- Vector (pET28a)
1320ng(220ng/µl)	- Insert (PCR product)
1µl	- T4 DNA Ligase
2µl	- Ligase Buffer
9µl	- Nuclease Free Water
20µl	- Total Volume

Insert

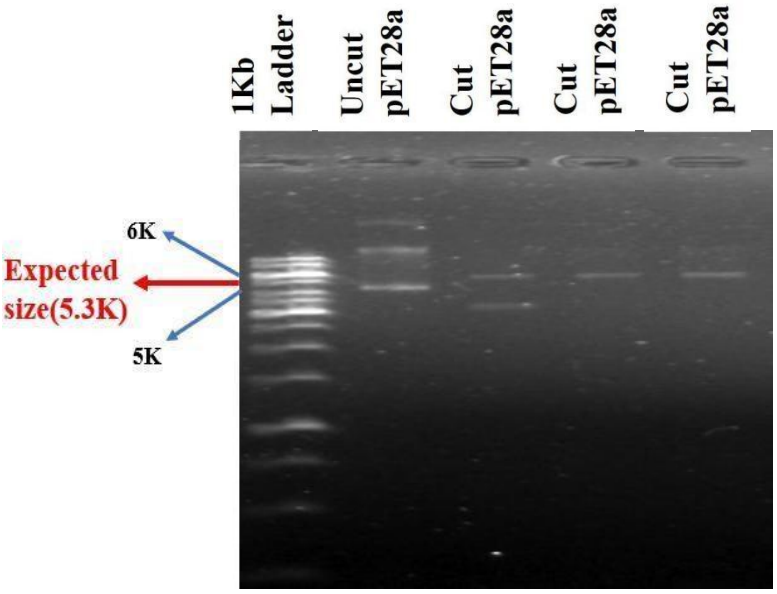


FigC.1.: Digested purified PCR product

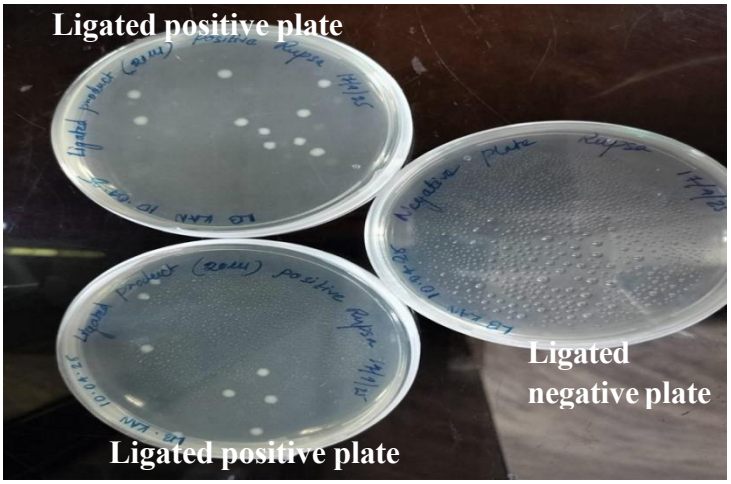
Vector



FigC.2.: pET-28a plate



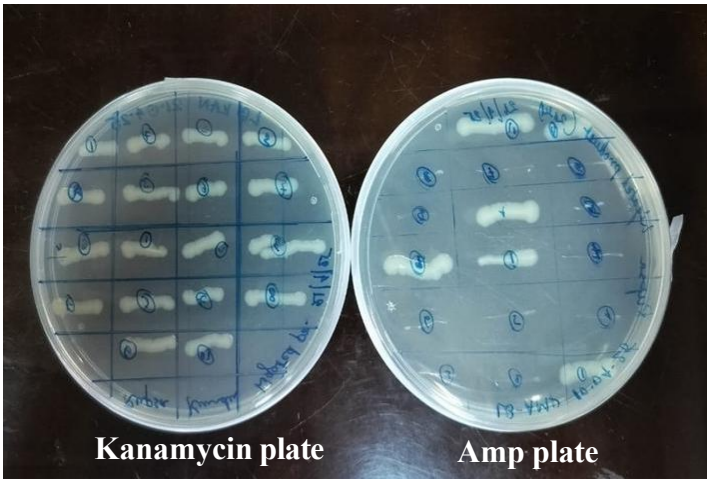
FigC.3.: Confirmation of pET28a



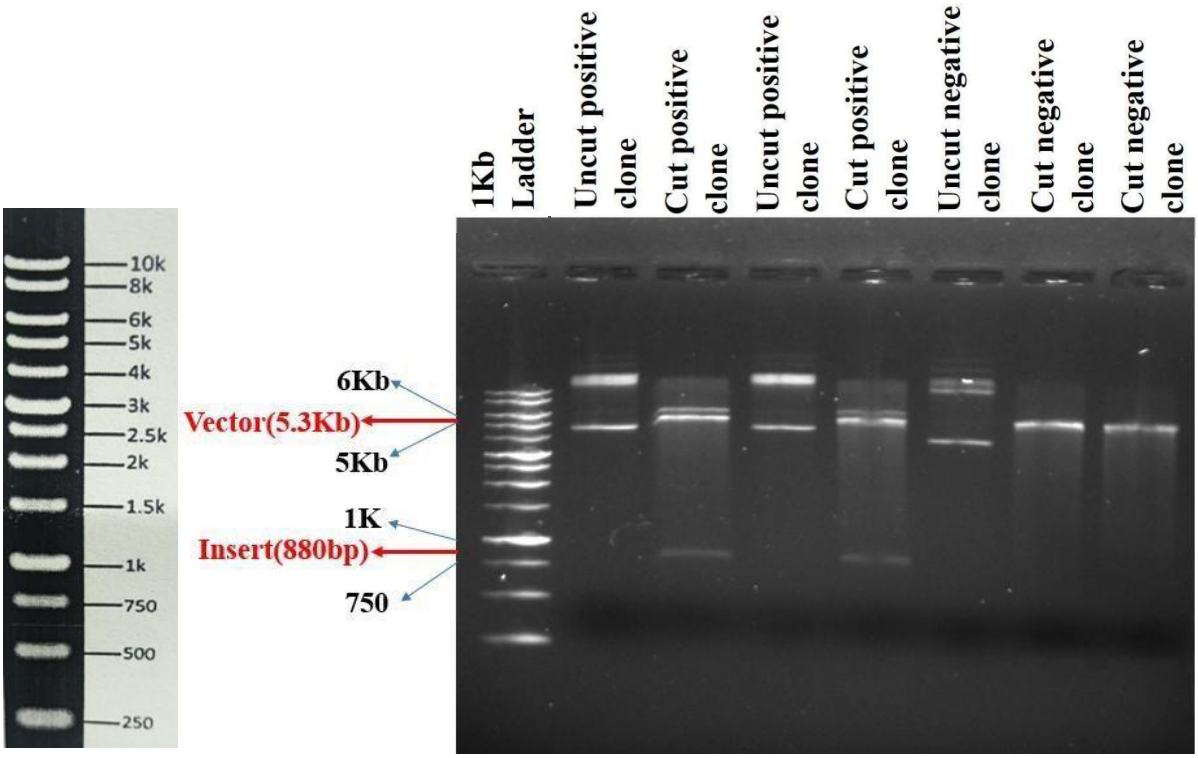
FigC.4.: Selection plate for ligated plasmid

D. DOUBLE SELECTION SCREENING:

Following double selection cloning, only colonies that survived on LB agar plates containing both ampicillin and kanamycin were considered successful recombinants. These colonies were further screened, and plasmid extraction followed by restriction digestion confirmed the presence of the insert, indicated by bands of expected sizes on agarose gel electrophoresis.



FigD.1.: Double selection on ampicillin plate



FigD.2.: Confirmation of ligated positive clone

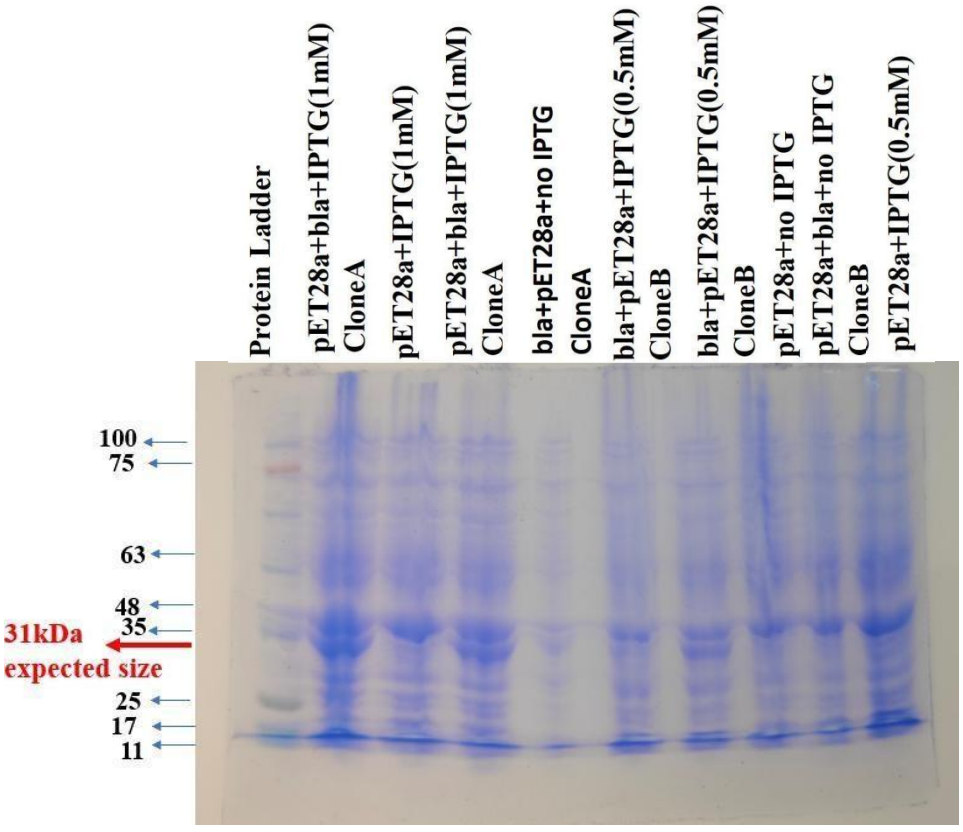
E. EXPRESSION OF β-LACTAMASE & SDS PAGE ANALYSIS:

Table E.1.a.: 12% Running gel (Resolving Gel) preparation

REAGENT	AMOUNT
30% Acrylamide- bis acrylamide	4ml
1.5M Tris-HCL (pH 8.8)	2.5ml
10% SDS	100µl (0.1ml)
10% APS	100µl (0.1ml)
TEMED	10µl (0.01ml)
Distilled Water	3.29ml
Total	10ml

TableE.2.b.: 5% Stacking gel preparation

REAGENT	AMOUNT
30% acrylamide-bis acrylamide	0.67ml
1M Tris-HCL (pH 6.8)	1.25ml
10% SDS	50µl (0.05ml)
10% APS	50µl (0.05ml)
TEMED	5µl (0.005ml)
Distilled Water	2.975ml
Total	5ml



FigE.1: Expression of β-lactamase

DISCUSSIONS

The present study aimed to develop an efficient bacterial expression system for the therapeutic production of β-lactamase. The bla gene encoding β-lactamase was successfully amplified and cloned it directly into the pET-28a expression vector. Under the direction of the T7 promoter, this approach compatibility with high level expression in *E. coli BL21 (DE3).*
The existence of the gene was confirmed by the production of a unique band of the anticipated size following PCR amplification of the bla gene using specially created primers with restriction enzyme sites (EcoRI & HindIII). Restriction digestion and subsequent ligation into the vectors were validated through gel electrophoresis and colony PCR, demonstrating successful cloning.

Propagation of the construct was made possible by transformation into *E. coli DH5α* for cloning and B*L21(DE3*) for expression. The pUC19 vector facilitated preliminary screening, while pET-28a ensured robust expression upon IPTG induction. SDS-PAGE analysis showed distinct bands corresponding to β-lactamase, indicating successful overexpression in the *BL21* host system. The expression level was notably higher post-induction, confirming the effectiveness of the T7 system in driving recombinant protein production.

Although enzymatic activity assays were not part of the current study, the structural integrity and molecular weight matched known β-lactamase profiles, suggesting successful expression. This system provides a promising platform for the large-scale production of β-lactamase, which may be further adapted for therapeutic or research purposes. Future work could include protein activity assays, kinetic characterization, and structural studies to fully evaluate the therapeutic potential of the expressed protein.

CONCLUSION

In this study, a bacterial expression system was successfully developed for the therapeutic production of β-lactamase. The bla gene was efficiently amplified from pUC19 and direct cloned into the pET-28a vector to enable high-level expression in *E. coli BL21 (DE3)*.

The use of T7 promoter-based expression in the pET-28a system, along with IPTG induction, resulted in effective overexpression of the β-lactamase protein. The presence of the target protein was confirmed by SDS-PAGE analysis, indicating that the expression system functioned as intended.

This project demonstrates the potential of using *E. coli* as a host for the recombinant production of therapeutic enzymes such as β-lactamase. The system developed here can serve as a foundation for future scale-up, functional assays, and potential therapeutic applications. Further studies involving purification optimization and enzymatic activity analysis will strengthen the applicability of this system in real-world biotechnological or pharmaceutical settings.

APPENDIX

Table1. Materials and reagents

Category	Item	Description
Bacterial strains	*E. coli DH5α*	Used for cloning of recombinant plasmids
	E. coli BL21	Used for protein expression
Plasmids	pUC19	High copy cloning vector containg bla gene
	pET-28a	Expression vector with T7 promoter and His-tag
Enzymes	Taq DNA polymerase	For PCR amplification
	EcoRI & HindIII	Restriction enzymes for digestion
	T4 DNA Ligase	For ligation of inserts into vectors
Buffers	LB broth & LB agar	For bacterial culture
	1X TAE buffer	For DNA electrophoresis
	Lysis Buffer	For protein extraction
Reagents	IPTG	Induce for protein expression
	Ampicillin, Kanamycin	Antibiotics for selection
	Agarose, Ethidium bromide	For gel electrophoresis

Table2. Instruments and Equipment

Equipment	Purpose
Thermocycler	For PCR amplification
Gel Electrophoresis	To analyze DNA fragments
UV transilluminator	For visualization of DNA gels
Incubator Shaker	For bacterial growth and expression

Spectrophotometer	To measure OD_{600} for cell density
Refrigerated Centrifuge	For pelleting cells and proteins
SDS-PAGE System	For protein separation
Sonicator	For cell lysis

Table3. List of primer

Primer	**Sequence (5'-3')**	**Restriction Site**
Forward primer	ATGAGTATTCAACATTTCCGTGTCG	EcoRI
Reverse primer	AGTAAACTTGGTCTGACAGTTACCA	HindIII

The primers were customed-designed to amplify the bla gene from pUC19 with added restriction sites for EcoRI and HindIII to facilitate directional cloning.

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ABSTRACT

A key component of contemporary biotechnology is the creation of recombinant proteins using bacterial expression systems, which provide scalable and reasonably priced platforms for therapeutic uses.. In the study, we developed a bacterial system for the expression of β-lactamase, an enzyme known not only for conferring antibiotic resistance but also for its emerging potential in therapeutic interventions, such as protecting gut microbiota during antibiotic treatment.

The bla gene encoding β-lactamase was selectively ampli ed from the pUC19 cloning vector using a pair of synthetic primers designed to include EcoRI and HindIII restriction sites, enabling directional cloning. Initial ampli cation attempts with supercoiled pUC19 DNA were unsuccessful, likely due to reduced primer accessibility. The target ~800bp segment was ampli ed ef ciently by PCR after pUC19 was digested to linear form. The puri ed PCR product was digested with appropriate restriction enzymes and ligated into the pET-28a expression vector, which provides a T7 promoter for high-level transcription and a His-tag for potential puri cation. The ligation product was transformed into E. coli BL21(DE3), a strain engineered for T7 polymerase- mediated expression. Transformants were selected on kanamycin agar plates and subsequently re-screened on ampicillin for dual-selection veri cation. By means of restriction enzyme digestion and plasmid isolation, recombinant colonies were veri ed. Con rmed clones were cultured, and protein expression was induced using 1mM IPTG at mid-log phase (OD600 0.6-0.8). After induction, cells were harvested and lysed, and the protein extract was denatured with Laemmli buffer at 95°C to unfold proteins. Successful expression was con rmed by SDS-PAGE analysis, which showed a clear band at about 31kDa, which is the molecular weight of β-lactamase. The project demonstrates the successful construction and functional veri cation of a recombinant expression system for β-lactamase in E. coli. The methodology used serve as a foundational approach for the production of therapeutic proteins in prokaryotic hosts, with future potential for puri cation, characterization, and downstream biomedical application.

KEYWORD

β-lactamase, Therapeutic protein, Recombinant expression, pUC19, pET-28a, E. coli BL21(DE3), PCR ampli cation, IPTG induction, SDS-PAGE, Molecular Cloning, Antibiotic Resistance.

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INTRODUCTION

Recombinant DNA technology has greatly revolutionised biotechnology by allowing microbial synthesis of therapeutic proteins. Among microbial hosts, Escherichia coli is extensively used due to its rapid proliferation, well-understood genetics, and ability to express diverse foreign proteins. Utilizing bacterial expression systems offers multiple bene ts, including scalability, low production costs, and ease of genetic manipulation. This study aims to establish a bacterial platform for the recombinant expression of β-lactamase, which serves both as a model enzyme and a potential therapeutic candidate.

β-lactamase is an enzyme capable of breaking down β-lactam antibiotics, such as penicillin and cephalosporin, rendering them ineffective. Although commonly associated with antibiotic resistance, recent ndings indicate that β-lactamase can be repurposed for therapeutic use. For instance, it may help preserve the gut microbiome during antibiotic treatment by neutralizing residual antibiotics in the gastrointestinal tract. Given this therapeutic potential, β-lactamase was selected as the target protein for expression in a bacterial host.

The gene encoding β-lactamase (bla) is naturally carried by the pUC19 plasmid—a frequently used vector in molecular cloning. In this work, the bla gene was ampli ed from pUC19 using specially designed primers that incorporated recognition sequences for the restriction enzymes EcoRI and HindIII. These locations were chosen in order to facilitate directional cloning into the pET-28a expression vector's multiple cloning site (MCS). To improve ampli cation ef ciency, pUC19 was linearized by restriction digestion prior to PCR, mitigating the limitations posed by supercoiled DNA.

In order to create cohesive ends that were compatible with the digested pET-28a vector, the bla gene was isolated and digested using EcoRI and HindIII after PCR. A T7 promoter for robust, inducible expression and an N-terminal 6xHis-tag for simplicity of puri cation are features of this expression plasmid. The digested vector and insert were ligated using T4 DNA ligase and transformed into E. coli BL21(DE3) cells, which are optimized for T7 promoter-driven expression and inducible by IPTG.

Transformants were selected on LB agar plates containing kanamycin, as the pET-28a vector confers resistance to this antibiotic. Colonies underwent plasmid isolation and further screening on ampicillin-containing plates to con rm the inclusion of the bla gene. Restriction digestion of the extracted plasmids was conducted to verify the presence and correct orientation of the insert.

Recombinant strains con rmed to carry the bla gene were cultured, and protein expression was induced using 1 mM IPTG at mid-log phase (OD600 ~0.6–0.8). After induction, bacterial pellets were

collected, lysed, and prepared for SDS-PAGE analysis. Laemmli buffer was used to denature the samples and heated at 95°C. A protein band corresponding to β-lactamase was observed at 31kDa, con rming successful expression.

In summary, this project demonstrates the successful development of a bacterial expression system for β-lactamase using E. coli BL21(DE3) and the pET-28a vector. This system not only provides a model for the production of recombinant therapeutic proteins but also lays the groundwork for future puri cation and functional characterization studies. The methodologies applied in this study, including primer design, directional cloning, and protein expression analysis, are foundational to molecular biology and biotechnological applications.

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LITERATURE REVIEW

When genetic engineering and molecular cloning techniques are combined, the eld of producing recombinant proteins has seen great breakthroughs. Among various host systems, Escherichia coli remains the most widely used organism for the production of recombinant proteins due to its simple cultivation, rapid growth, and well-studied genetics. The ability to express cloned genes in E. coli has provided an essential platform for producing therapeutic, industrial, and research-grade proteins. One of the fundamental aspects of recombinant protein production involves the use of expression vectors that can ef ciently regulate gene transcription and translation. The pET-28a system, is a widely utilized expression vector that contains the T7 promoter for high-level expression and a 6x His-tag for easy protein puri cation through nickel af nity chromatography. A host strain such as E. coli BL21(DE3), which carries the T7 RNA polymerase gene under lac promoter control, is necessary for this approach. Induction with isopropyl β-D-1-thiogalactopyranoside (IPTG) triggers the expression of the gene cloned downstream of the T7 promoter.

β-lactamase, the target protein in this project, is a bacterial enzyme known for its ability to hydrolyze beta-lactam antibiotics, thereby conferring resistance to the producing organism. While traditionally associated with antimicrobial resistance, β-lactamase has garnered interest for its possible therapeutic applications. Studies have suggested that orally administered β-lactamase can inactivate antibiotics in the gut, potentially protecting commensal microbiota from disruption during antibiotic therapy (Ubeda et al., 2013). This functional versatility makes β-lactamase an interesting candidate for recombinant production and further functional exploration also for screening of β-lactamase inhibitors. The source of the β-lactamase gene in this study is the pUC19 plasmid, a high-copy-number cloning vector commonly used in molecular biology laboratories. It has the bla gene, which confers ampicillin resistance and encodes β-lactamase. The high-copy nature of pUC19 ensures ample quantities of plasmid DNA for downstream applications such as gene ampli cation.

Molecular cloning relies heavily on the polymerase chain reaction (PCR) for gene ampli cation. In this project, primers were designed with restriction enzyme recognition sites for EcoRI and HindIII to facilitate directional cloning. Compatibility between the ampli ed gene and the expression vector is ensured by include restriction sites in primers. Linearization of supercoiled plasmid DNA prior to PCR has been shown to improve ampli cation ef ciency, as it provides better accessibility for the DNA polymerase to the target region.

Following ampli cation and digestion, the gene insert and vector are ligated using T4 DNA ligase. This enzyme catalyzes the formation of phosphodiester bonds between adjacent nucleotides, thereby

creating a stable recombinant plasmid. The ligated product is then transformed into chemically competent E. coli cells, such as BL21(DE3), for propagation and expression. Transformation ef ciency and plasmid integrity are veri ed using antibiotic selection and plasmid isolation followed by restriction digestion analysis.

Upon con rming the presence of the recombinant construct, the transformed cells are cultured and induced with IPTG to initiate protein expression. Protein synthesis results from the transcription of the target gene by the T7 RNA polymerase during induction. The expressed proteins are typically harvested from the cell pellet, denatured using Laemmli buffer, and analyzed using SDS-PAGE. This technique separates proteins based on molecular weight, allowing visualization of the expressed β-lactamase as a distinct band at approximately 31kDa. Previous studies have established the ef ciency of E. coli systems for expressing a variety of therapeutic proteins, including β-lactamase. Research by (Banerjee et al. 2012) demonstrated successful expression and puri cation of recombinant β-lactamase, highlighting the reliability of the pET-28a system and the importance of expression condition optimization. These ndings provide a strong foundation for using bacterial systems in both research and therapeutic protein production.

In conclusion, the literature supports the selection of E. coli as a host organism, the use of pET-28a as an expression vector, and the utility of β-lactamase as a model therapeutic protein. The integration of these components in the present study is well-founded and represents a standard yet effective approach in recombinant protein biotechnology.

Plagiarized Sources

The source of the **β**-lactamase gene in this study is the pUC19 plasmid, a high-copy-number cloning vector commonly used in molecular biology laboratories.
https://www.neb.com/en/products/n3041-puc19-vector%3Fsrsltid%3DAfmBOoqZqtmNs2YDjLwSpA59iQEyhbxAyvMLgmgLOszT9av2-tgwHqKy

Following ampli cation and digestion, the gene insert and vector are ligated using T4 DNA ligase.
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coli cells, such as BL21(DE3), for propagation and expression.
https://pmc.ncbi.nlm.nih.gov/articles/PMC9968953

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METHODOLOGY

1. PRIMER DESIGNING PROCEDURE:

• The β-lactamase gene (bla gene) was ef ciently cloned from pUC19 and pET-28a was used for expression by a meticulously planned primer creation approach. The NCBI primer designing tool was utilized to guarantee speci city and ef ciency. The initial step consisted of choosing the target gene responsible for ampicillin resistance, which produces beta-lactamase. The forward primer was selected from a previously published study work “PNAS March 19,2002 vol. 99 no. 6 3469-3474”. Forward primer is ‘ATGAGTATTCAACATTTCCGTGTCG’. I looked for a reverse primer that would complement and bind at the precise intended site, using NCBI primer designing tool. To enhance the cloning process, EcoRI and HindIII restriction sites were added to the 5’ ends of the forward and reverse primers, respectively. This modi cation guaranteed that, following digestion, the gene could be inserted in the correct orientation into the multiple cloning site (MCS) of the vector without compromising critical sequence. After incorporating the restriction sites, the primers were ne-tuned for speci city, melting temperature (Tm), and GC content to facilitate effective binding and ampli cation during PCR. The GC content was kept between 40% and 60% to promote stable hybridization, while the melting temperature was set between 55°C and 65°C to limit primer-dimer formation and ensure reliable ampli cation. The primers were crafted to have lengths ranging from 18 to 25 base pairs, striking a balance between speci city and ef ciency. To verify their accuracy, the primers underwent testing with Snap Gene, where their binding locations and the existence of the restriction sites were con rmed. This procedure was crucial in ensuring that the primers would amplify the intended region without causing any unintended secondary structures or mismatches.After successful validation, the primers were obtained and dissolved in nuclease-free water to achieve a stock concentration of 100µM, with a working concentration of 10µM utilized in PCR reactions.

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4. PCR AMPLIFICATION:

After designing speci c primers with EcoRI and HindIII restriction sites, gene ampli cation was performed to isolate the beta-lactamase (ampicillin resistance) gene from the pUC19 plasmid. The initial PCR attempt using the intact pUC19 plasmid as the template did not yield successful ampli cation. This may have been due to secondary structures or inaccessibility of the target region within the supercoiled plasmid.

To overcome this issue, the pUC19 plasmid was rst digested with EcoRI and HindIII to linearize the DNA and expose the gene of interest. This linearized plasmid was then used as a template for PCR, which successfully resulted in ampli cation of the expected ~880 bp beta-lactamase gene. The PCR product was visualized on agarose gel, con rming the correct band size and the success of the ampli cation step.

Table 4.1: PCR cycle

step Temperature Time

Initial Denaturation 95°C 5 min

Denaturation 95°C 30 sec

Annealing 60°C 30 sec

Extension 72°C 53 sec

Final Extension 72°C 10 min

Hold 25°C 1min

5. PREPARATION OF INSERT:

After con rming successful ampli cation, the PCR product containing the bla gene was puri ed using a PCR puri cation kit to remove primers, nucleotides, enzymes, and salts. The puri ed product was then digested with EoRI and HindIII restriction enzymes to generate compatible ends for cloning. The digestion mixture was incubated under optimal conditions (typically at 37°C for 1 hour). Following digestion, the product was again puri ed using a PCR puri cation kit to isolate the digested fragment.

This puri ed, double digested insert was ready for ligation into the expression vector.
Flowchart

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6. PREPARATION OF EXPRESSION VECTOR (pET-28a):

The pET-28a vector was propagated in E. coli BL21(DE3) cells and plasmid DNA was extracted following by the previous method. In order to produce compatible sticky ends for ligation with the insert, the vector was digested with EcoRI and HindIII. The linearized vector was puri ed using a PCR puri cation kit same as before.

7. SUBCLONING INTO pET28a:

T4 DNA ligase was used to ligate the digested pET-28a vector and the puri ed, digested bla insert. The ligation reaction was incubated at optimal conditions, and the product was transformed into competent E. coli BL21(DE3) cells. Transformations were selected on kanamycin-resistant LB agar plates.

8. DOUBLE SELECTION SCREENING:

Colonies that grew on kanamycin plates were re-plated on ampicillin-resistant plates. Growth on both antibiotic plates con rmed the successful integration of the bla-gene. Positive colonies were selected for plasmid isolation.

9. CONFIRMATION OF CLONING:

Plasmid DNA from positive colonies was digested with EcoRI and HindIII to verify the presence of the insert. Gel electrophoresis revealed two bands, corresponding to the vector backbone (~5.3kb) and the insert (~880bp).

10. PROTEIN EXPRESSION VIA IPTG INDUCTION & SDS-PAGE ANALYSIS:

In this experiment, E. coli BL21(DE3) cells carrying the recombinant pET-28a plasmid containing the bla gene were used to express proteins. The bacterial culture was grown in LB broth with both kanamycin and ampicillin until it reached an optical density (OD_{600}) of approximately 0.6–0.8, indicating the mid-log phase of growth. At this stage, 1 mM and 0.5 mM IPTG were administered to activate the expression of the β-lactamase gene regulated by the T7 promoter.

Following induction, the culture was incubated for 4 hours to allow suf cient protein production. Cells were then harvested by centrifugation, and the resulting pellet was resuspended in Laemmli buffer. The sample was heated at 95°C to denature the proteins and ensure uniform unfolding before analysis.

The expressed protein was analyzed by SDS-PAGE, a technique that separates proteins based on molecular weight. A clear and distinct band corresponding to approximately 31kDa was observed, which matched the expected size of β-lactamase. This result con rmed that the bla gene was successfully expressed in the bacterial system and that the recombinant protein was present in the cell lysate.

Plagiarized Sources

Positive colonies were selected for plasmid isolation.
https://pmc.ncbi.nlm.nih.gov/articles/PMC6252260

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White colonies appeared on LB agar plates containing ampicillin, indicating successful transformation of E. coli DH5α with the pUC19 plasmid. No colonies were observed on the control plate. Plasmid was extracted from chosen colonies and underwent restriction digestion.. Agarose gel electrophoresis showed bands corresponding to the expected size of pUC19, con rming the presence of the plasmid.
FigA.1: pUC19 plate
FigA.2.: con rmation of pUC19 plasmid
FigA.3.: 1% Agarose Gel
B.PCR AMPLIFICATION:

PCR reagent Amount
DNTP 2µl
PCR buffer(10X) 2.5µl
Reverse Primer(10µM) 0.5µl
Forward Primer(10µM) 0.5µl
Taq polymerase 0.25µl
Template DNA (digested DNA) 1µl (16.9ng/µl)
Nuclease Free water 18.25µl
Total 25µl
Reagent Amount
pUC19 DNA 2µl (338ng/µl)
Enzyme (EcoRI & HindIII) 0.5µl
Tango Buffer 2µl
Nuclease Free Water 15µl
Total 20µl
FigB.3.: Initial failure due to supercoiled plasmid
FigB.4.: Successful ampli cation (Expected size ~880bp)
C. LIGATION
Insert (PCR product) preparation:

1.Incubate 780 ng of PCR-ampli ed DNA with EcoRI and HindIII for 2 hours at 37°C.
780ng(130ng/µl) - pUC19 DNA
1µl - EcoRI enzyme (10 units/µl)
1µl - HindIII enzyme (10 units/µl)
3µl - Tango buffer (10X)

19µl - Nuclease Free Water
30µl - Total Volume

2. Purify the digested plasmid using PCR puri cation kit.

Vector(pET28a) preparation:

1. Digest 1128ng(188.2ng/µl) of pET28a with EcoRI & HindIII for 2 hours at 37°C.
1128ng(188.2ng/µl) - pET28a DNA
1µl - EcoRI (10 units/µl)
1µl - HindIII (10 units/µl)
3µl - Tango Buffer (10X)
19µl - Nuclease Free Water
30µl - Total Volume
2. Purify the digested pET28a plasmid using PCR puri cation kit.

Set up ligation reaction and incubated at 16°C for overnight.
700ng(350ng/µl) - Vector (pET28a)
1320ng(220ng/µl) - Insert (PCR product)
1µl - T4 DNA Ligase
2µl - Ligase Buffer
9µl - Nuclease Free Water
20µl - Total Volume
FigC.1.: Digested puri ed PCR product
FigC.3.: Con rmation of pET28a
FigC.4.: Selection plate for ligated plasmid

D. DOUBLE SELECTION SCREENING:
Following double selection cloning, only colonies that survived on LB agar plates containing both ampicillin and kanamycin were considered successful recombinants. These colonies were further screened, and plasmid extraction followed by restriction digestion con rmed the presence of the insert, indicated by bands of expected sizes on agarose gel electrophoresis.
FigD.1.: Double selection on ampicillin plate
FigD.2.: Con rmation of ligated positive clone

E. EXPRESSION OF β-LACTAMASE & SDS PAGE ANALYSIS:

REAGENT AMOUNT
30% Acrylamide- bis acrylamide 4ml
1.5M Tris-HCL (pH 8.8) 2.5ml
10% SDS 100µl (0.1ml)
10% APS 100µl (0.1ml)
TEMED 10µl (0.01ml)
Distilled Water 3.29ml
Total 10ml
REAGENT AMOUNT
30% acrylamide-bis acrylamide 0.67ml
1M Tris-HCL (pH 6.8) 1.25ml
10% SDS 50µl (0.05ml)
10% APS 50µl (0.05ml)
TEMED 5µl (0.005ml)
Distilled Water 2.975ml
Total 5ml

FigE.1: Expression of β-lactamase

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DISCUSSIONS

The present study aimed to develop an ef cient bacterial expression system for the therapeutic production of β-lactamase. The bla gene encoding β-lactamase was successfully ampli ed and cloned it directly into the pET-28a expression vector. Under the direction of the T7 promoter, this approach compatibility with high level expression in E. coli BL21 (DE3).
The existence of the gene was con rmed by the production of a unique band of the anticipated size following PCR ampli cation of the bla gene using specially created primers with restriction enzyme sites (EcoRI & HindIII). Restriction digestion and subsequent ligation into the vectors were validated through gel electrophoresis and colony PCR, demonstrating successful cloning.

Propagation of the construct was made possible by transformation into E. coli DH5α and BL21(DE3). The pUC19 vector facilitated preliminary screening, while pET-28a ensured robust expression upon IPTG induction. SDS-PAGE analysis showed distinct bands corresponding to β-lactamase, indicating successful overexpression in the BL21 host system. The expression level was notably higher post-induction, con rming the effectiveness of the T7 system in driving recombinant protein production.

Although enzymatic activity assays were not part of the current study, the structural integrity and molecular weight matched known β-lactamase pro les, suggesting successful expression. This system provides a promising platform for the large-scale production of β-lactamase, which may be further adapted for therapeutic or research purposes. Future work could include protein activity assays, kinetic characterization, and structural studies to fully evaluate the therapeutic potential of the expressed protein.

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CONCLUSION

In this study, a bacterial expression system was successfully developed for the therapeutic production of β-lactamase. The bla gene was ef ciently ampli ed and cloned into pUC19 for initial con rmation, and subsequently subcloned into the pET-28a vector to enable high-level expression in E. coli BL21 (DE3).

The use of T7 promoter-based expression in the pET-28a system, along with IPTG induction, resulted in effective overexpression of the β-lactamase protein. The presence of the target protein was con rmed by SDS-PAGE analysis, indicating that the expression system functioned as intended.

This project demonstrates the potential of using E. coli as a host for the recombinant production of therapeutic enzymes such as β-lactamase. The system developed here can serve as a foundation for future scale-up, functional assays, and potential therapeutic applications. Further studies involving puri cation optimization and enzymatic activity analysis will strengthen the applicability of this system in real-world biotechnological or pharmaceutical settings.

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